

Myrmecophilous organs and ant association of the larvae of *Tongeia fischeri* (Eversmann, 1843) (Lycaenidae: Polyommagini)

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Abstract Butterflies in the family Lycaenidae have for a long time been known for their association with ants. These associations can be facultative or obligate, ranging from mutualism to parasitism and have strongly influenced the development of the larval morphology. Here, we describe myrmecophilous organs of the larva of *Tongeia fischeri* and compare them to some other lycaenid species. The larva possesses three types of myrmecophilous organs, namely pore cupola organs (PCOs), tentacle organs (TOs) and a dorsal nectary organ (DNO). During field surveys in Kyushu, Japan, seven attendant ants were identified as follows: *Ochetellus glaber*, *Lasius productus*, *Cardiocondyla nuda*, *Crematogaster nawai*, *Pheidole fervens*, *Tetramorium bicarinatum* and *Tetramorium tsushimae*. This nonspecific ant-association of *T. fischeri* suggests that the butterfly is a facultative myrmecophilous species.

Key words Formicidae, Lycaenidae, mutualism, myrmecophily, myrmecophilous organs, scanning electron microscopy.

Introduction

The family Lycaenidae (Lepidoptera: Papilionoidea), the second largest among true butterfly families, is well known for the association of many of its species with ants (Hymenoptera: Formicidae), termed myrmecophily (Fiedler, 1991). About 75 percent of the known, full or partial, life histories of members of the family have been recorded with ant-association, ranging from mutualism to parasitism (Pierce *et al.*, 2002). These symbiotic interactions fall into two broad categories: obligate, where the survival of immature stages under field conditions is dependent on specific attendant ants; and facultative, where the larvae have often associated with nonspecific ant species of various groups, ranging from congeners to different genera or subfamilies, and do not require ants for survival under field or laboratory conditions (Fiedler, 1991; Pierce *et al.*, 2002). The strong associations with ants have influenced the development of larval morphology. The larvae in Lycaenidae usually have a thick cuticle, perhaps 20–30 times thicker than that of other lepidopterous larvae, and a small, retractable head that can hide under a sclerotized prothoracic plate for defense against ant bites (Malicky, 1970). In more specialized adaptations, lycaenid larvae possess sets of chemical signaling and acoustic signaling organs that serve specific functions in their interactions with ants (Fiedler, 1991; Pierce *et al.*, 2002; Yago, 2003b; 2010).

Fischer's blue, *Tongeia fischeri* (Eversmann, 1843), is a small butterfly in the family Lycaenidae. The butterfly is

characterized by a distinct black color on the upperside of the wings, pale grey ground-color with small black dots on the undersides of both wings, orange tinged spots near the outer edge on the underside of the hindwing and a pair of minute tails. It is widely distributed from eastern Europe to northeastern Asia (Yakovlev, 2003). Adult butterflies fly actively near rocky steppes, brook slopes of rivers, brook valleys, river banks, open forests or coastal rocks, where their larval host plants grow. Larvae are generally greenish with pink dorsal and lateral lines; some are milky white with a red tint. They can be found on or near the host plant where they live and feed inside. Larvae hibernate inside or near the host plants during winter (Fukuda *et al.*, 1984).

Although the immature stages and myrmecophily of *T. fischeri* have been previously reported elsewhere (Iwase, 1954; Fukuda *et al.*, 1984; Fiedler, 1991), the detailed structure of the myrmecophilous organs and the classification of the attendant ants have been poorly studied. The myrmecophilous structures of the species have been reported only by Yago (2003a), but this information has not been widely disseminated. Additionally, the attendant ants have been recorded by Fukuda *et al.* (1984), Watanabe (1987), Yago (2007), and Nishio (2012). However, most species of ants in these records were unidentified.

Here, we provide detailed information on myrmecophilous organs found in the late instar larvae of *T. fischeri* with the aid of scanning electron microscopy (SEM) and identify attendant ants collected from Kyushu, Japan.

Table 1. Localities and host plants researched in the present study.

No.	Locality name	Coordinates	Host plant
1	Tojushi, Tsushima, Nagasaki	34.37049N 129.28436E	<i>Orostachys japonica</i> (Maxim.)
2	Are, Tsushima, Nagasaki	34.16185N 129.11543E	<i>Orostachys japonica</i> (Maxim.) <i>Sedum japonicum oryzifolium</i> (Makino) H. Ohba
3	Waniura, Tsushima, Nagasaki	34.41405N 129.26218E	<i>Orostachys japonica</i> (Maxim.)
4	Futaojima, Yamaguchi	34.06011N 130.47156E	<i>Orostachys malacophylla</i> var. <i>iwarenge</i> (Makino)
5	Ainoshima, Fukuoka	33.45357N 130.22333E	<i>Sedum japonicum oryzifolium</i> (Makino) H. Ohba
6	Odomo, Karatsu, Saga	33.54272N 129.91376E	<i>Sedum japonicum oryzifolium</i> (Makino) H. Ohba
7	Yamakuni, Nakatsu, Oita	33.24093N 131.02374E	<i>Orostachys japonica</i> (Maxim.)
8	Yabakei, Nakatsu, Oita	33.22538N 131.11130E	<i>Orostachys japonica</i> (Maxim.)
9	Shishijima, Kagoshima	32.16005N 130.14337E	<i>Orostachys japonica</i> (Maxim.)

Materials and methods

Larvae of *T. fischeri* and their attendant ants were collected from nine localities during research trips in September 2009 and October 2011 in Kyushu and adjacent islands, Japan (localities are shown in Table 1). Late instar larvae were collected and brought back alive to the Laboratory of Systematic Zoology, Kyoto University. Two larvae were subjected to SEM for the study of the microstructure of myrmecophilous organs. Specimens were placed overnight in glutal aldehyde, washed with phosphate buffer, post-fixed with 1% osmium tetroxide, dehydrated through a series of acetone baths, critical-point dried, and sputter-coated with gold prior to examination in a JSM-5800 scanning electron microscope located at the laboratory of Developmental Biology, Kyoto University.

Attendant ants of *T. fischeri* were collected by hand and kept individually in 99.5 % ethanol with full records of locality name, date taken and coordinates. The ant voucher specimens are deposited at the Chulalongkorn University Museum of Natural History, Chulalongkorn University, Thailand. Identification of attendant ants was made by the second author, and followed the pictorial keys and descriptions provided in the book *Ants of Japan* (Imai *et al.*, 2003).

Results and Discussion

1. Myrmecophilous organs

The following three types of myrmecophilous organs were observed in the late instar larvae of *T. fischeri*.

Dorsal nectary organ (DNO; Figs 1a, c). DNO of *T. fischeri* larvae is visible as a single large organ on the dorsal portion of the seventh abdominal segment (Fig. 1a). It is apparently elastic, lightly sclerotized with a major transverse slit and two circular openings on each side. A dense scattering of pore cupola organs (PCOs) is located asymmetrically around it (Fig. 1c). The function of this DNO is to secrete

droplets of nutritive liquid containing amino acids and sugars (mainly sucrose and glucose) as an exocrine gland when stimulated by ants (Maschwitz *et al.*, 1975; Pierce, 1983; Daniels *et al.*, 2005). Figure 2c shows a worker ant of *Ochetellus glaber* drinking the nutritious secretion from the DNO of a *T. fischeri* larva. Lycaenid larvae produce these secretions as nutritive rewards to the attending ants in return for protection gained from natural enemies (Pierce and Mead, 1981). Under threat, they produce more such fluid and are twice as much tended by their protective ants (Agrawal and Fordyce, 2000). Comparison among lycaenid species shows that secretions rich in amino acids are found in obligate relationships, whereas facultative relationships rely on carbohydrate secretions (Daniels *et al.*, 2005). Although the DNO has been noted as an important organ for maintaining interaction between lycaenid larvae and ants (Fiedler *et al.*, 1996; Pierce *et al.*, 2002), it is not always present in all species of lycaenid larvae that are found in close association with ants (e.g. Yago, 2003a; Okubo *et al.*, 2009; Oliver and Stein, 2011). The presence of a DNO is widely observed in lycaenid larvae and probably is a primitive condition especially in the Lycaeninae. However, the DNO in some species of myrmecophilous groups appears to be in a stage of reduction, and this reduction has occurred repeatedly throughout the family (Malicky, 1970; Fiedler, 1991; Yago, 2003a; 2010).

Tentacle organs (TOs; Figs 1a, b, Fig. 2b). A pair of eversible tentacle organs, with a cylindrical structure when fully everted and approximately 20 plumose setae arranged around their tip, are located on each side of the eighth abdominal segment. The everted TOs are shown in Fig. 2b and at higher magnification in Fig. 1b. Each of the TOs is provided with a single retractor muscle originating from the ventral body wall and attached at the tip of the organ. TOs from either side are everted spontaneously, independent from each other, by an increase in hemolymph pressure (Vegliante and Hasenfuss, 2012). These organs are most

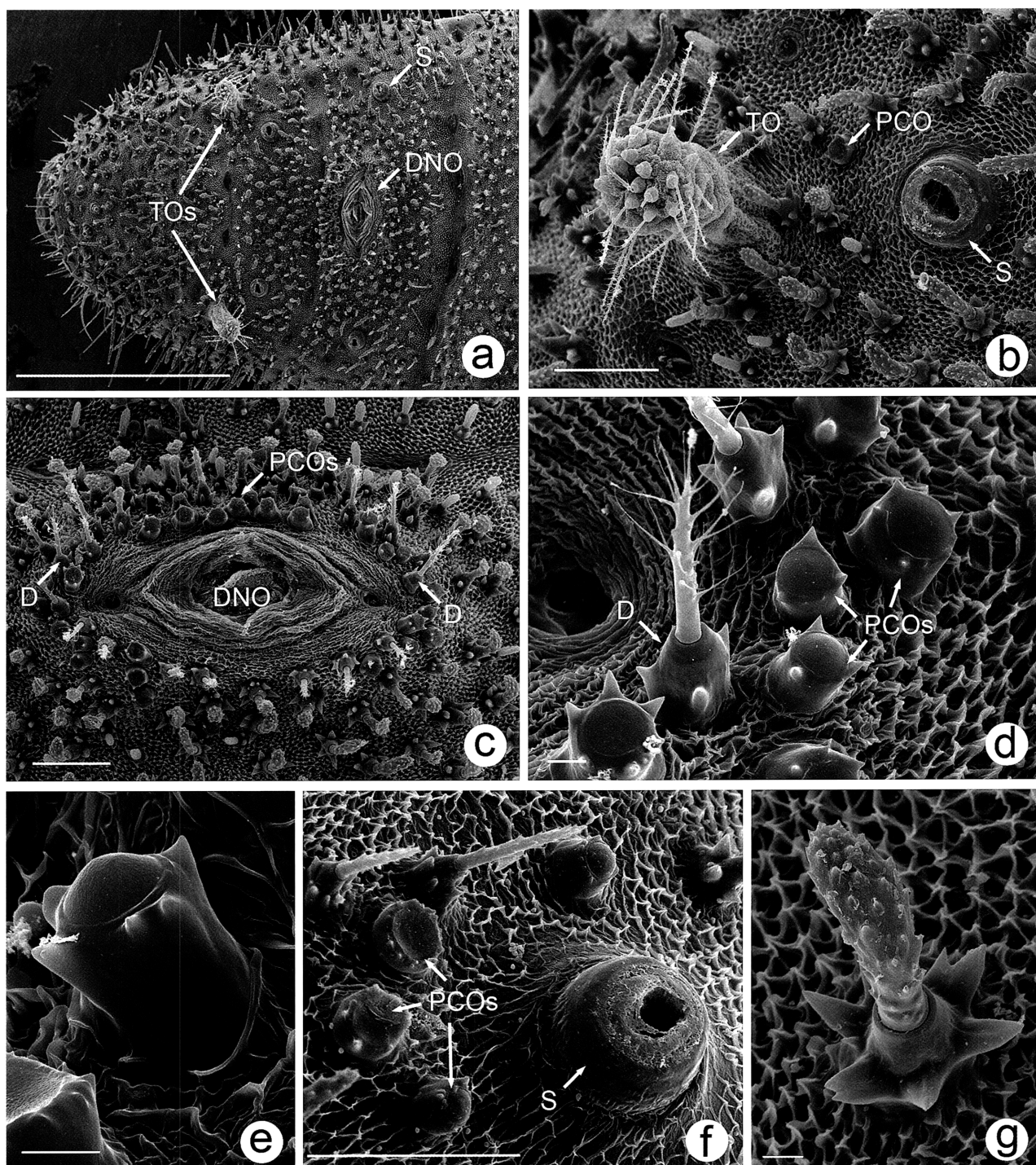


Fig. 1. Myrmecophilous organs of *Tongeia fischeri* larva. a) overview of the sixth to tenth abdominal segments with paired tentacle organs (TOs), dorsal nectary organ (DNO) and spiracles (S), scale bar = 1 mm; b) tentacle organ (TO), pore cupola organ (PCO) and spiracle (S) on the eighth abdominal segment, scale bar = 100 μ m; c) dorsal nectary organ (DNO) surrounded by a cluster of pore cupola organs (PCOs) and dendritic setae (D), scale bar = 100 μ m; d) dendritic seta (D) and pore cupola organs (PCOs) near dorsal nectary organ, scale bar = 10 μ m; e) pore cupola organ, scale bar = 10 μ m; f) spiracle (S) and pore cupola organs (PCOs), scale bar = 100 μ m; g) stellate based seta, scale bar = 10 μ m.

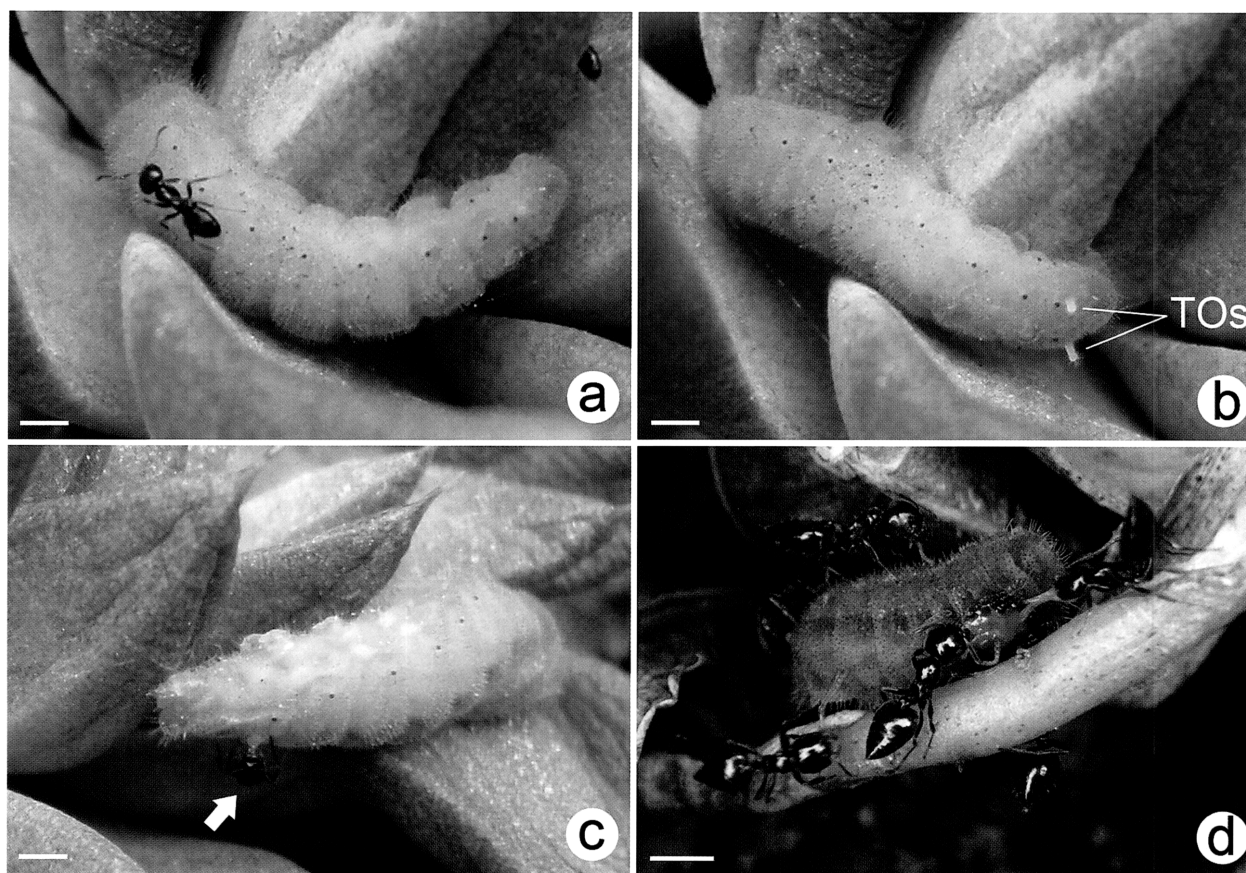


Fig. 2. Late instar larvae of *Tongeia fischeri* and its attendant ants. a) larva tended by a worker ant of *Ochetellus glaber* on the host plant *Orostachys japonica* (Maxim.) (Crassulaceae); b) larva everting tentacle organs (TOs); c) worker ants of *O. glaber* drinking nutritious secretion from dorsal nectary organ (DNO) of the larva (white arrow); and d) wounded larva overwhelmed and attacked by worker ants of *Crematogaster nawai* on the stem of a host plant *Sedum japonicum oryzifolium* (Makino) H. Ohba (Crassulaceae). Scale bars = 1 mm. a-c) photos by T. Nakano (Waniura, Tsushima, Nagasaki) and c) by E. Jeratthitikul (Ainoshima, Fukuoka).

frequently everted when larva-ant interaction commences, when the caterpillars are disturbed or stimulated by ants, or when the larvae are travelling back from feeding to resting places (Claassens and Dickson, 1977; AxéN *et al.*, 1996; Fiedler *et al.*, 1996). Although they may secrete volatile chemical compounds which may mimic short-range alarm pheromones of the ants (Claassens and Dickson, 1977; Henning, 1983; Fiedler *et al.*, 1996), the precise function of the organs is still unclear.

Pore cupola organs (PCOs; Figs 1b-f). Each PCO is a single-cell, columnar epidermal organ surrounded by four to eight short triangular projections on the lateral wall and covered with a convex/concave lens-shaped sieve plate at the top. Some are located on a prominent spinose surface, and others on an irregularly shaped surface (Figs 1d, e). They were found scattered over dorsal and lateral body regions of the larvae but with more clustered near the

opening of the DNO (Figs 1c, d) and around spiracles (Fig. 1f). The areas that have this accumulation of PCOs have been noted for great attention by attendant ants (Malicky, 1970). The PCOs may secrete volatile substances, such as amino acids and hydrocarbons, which are similar to those employed by ants to recognize conspecifics or their broods (Malicky, 1970; Pierce, 1983; Akino *et al.*, 1999; Yamaoka, 2000; Hojo *et al.*, 2008). The shape of PCOs varies among the examined lycaenid larvae of different species, especially on the basal part of the organ. Some of variants are hemispherical base in *Lysandra coridon*, hemispherical base with a ring of caruncles in *Lysandra bellargus*, and irregularly shaped bases in *Maculinea arion* (Kitching and Luke, 1985). The larvae of *Cupido minimus*, which is closely related to *Tongeia* and *Everes* species, possess PCOs that look apparently similar to those in *T. fischeri* (Baylis and Kitching, 1988). The PCOs are found almost universally on lycaenid larvae,

one exception being *Liphyra brassolis*, whereas TOs and/or DNO are absent in some taxa throughout the family Lycaenidae. For examples, butterflies in the subfamily Miletinae usually lack both a DNO and TOs, but those in the genus *Aslauga* lack only the DNO (Fiedler, 1991; Pierce *et al.*, 2002).

Additional structures (Figs 1c, d). The other structures that may be important in maintaining interactions between *T. fischeri* larvae and attendant ants, found in the present study, are dendritic setae. The setae are erect, tree-like in appearance, with long filamentous lateral processes, and arise from a prominent stellate socket (Fig. 1d). In *T. fischeri* larvae, they are restricted to the lateral margin of the DNO (Fig. 1c). Dendritic setae occur on larvae and/or pupae of many members of the Lycaenidae (Ballmer and Pratt, 1988; Yago 2003a). The setae vary in shape, density and distribution among lycaenid species (Kitching and Luke, 1985; Ballmer, 1991), and have been used as informative characters for larval identification (Ballmer and Pratt, 1988). The dendritic setae are known as mechanoreceptors and their function is considered to be as a sensory organ used to recognize attendant ants (Tautz and Fiedler, 1992). Kitching (1983) has noted that dendritic setae in *Jalmenus evagoras* were directed inwards over the opening of the DNO when the organ was inactive but moved out and became erect when a droplet is exuded by the organ. These setae hold the droplet in place until attendant ants collected it. Among twelve Californian species, members of the subfamily Lycaeninae, which lack both DNO and TOs, only the four which possess dendritic setae have been found associated with ants, and greater abundance of dendritic setae coincided with greater ant attendance (Ballmer, 1991). However, two butterflies in the tribe Polyommataini, *Brephidium exile* and *Hemiargus isola*, in which dendritic setae are not present, have also shown strong attendance by ants (Ballmer and Pratt, 1988). These reports suggest that the degree of ant attendance may depend on the concerted function of several involved organs, not merely on one organ.

In addition to myrmecophilous organs, *T. fischeri* larvae bear many erect, apically curved setae with sclerotized stellate sockets, throughout the dorsal surface of the thorax and abdomen (Fig. 1g). These setae with stellate or crown-like sockets are commonly found in most species of Polyommataini and other members of the genus *Tongeia* (Ballmer and Pratt, 1988; Yago, 2003a; Jerathtitikul *et al.*, 2011).

2. Attendant ants and myrmecophily

Seven species of ants from three subfamilies were in attendance on *T. fischeri* larvae: *Ochetellus glaber*, *Lasius productus*, *Cardiocondyla nuda*, *Crematogaster*

nawai, *Pheidole fervens*, *Tetramorium bicarinatum* and *Tetramorium tsushima*. As far as we can determine, only *Formica japonica* and *T. tsushima* have previously been reported as attendant ants on *T. fischeri* (Nishio, 2012). Therefore, six of these seven listed species may be recorded for the first time.

Of the seven ant species collected in our surveys, *C. nawai* formed the majority of the observations. It was found in five of the nine collecting sites, both in coastal and inland habitats (Table 2). This ant species is abundant near seashores and generally nests under stones (Imai *et al.*, 2003), which is the same habitat as utilised by *T. fischeri* larvae. This is no doubt the reason why *T. fischeri* larvae have been often found tended by the workers of *C. nawai*. Other *Crematogaster* species also have been noted to tend lycaenid larvae worldwide and this genus seems to be one of the most important partners for Lycaenidae, since 89 *Crematogaster* species are known for association with Lycaenidae and 41 of these exhibit obligate associations (Fiedler, 1991).

Late instars of *T. fischeri* were always found tended by one to three ant workers (Figs 2a, c); but early instars, prepupae and pupae were rarely found with ants. On one occasion, a single pupa was found tended by two workers of *C. nawai* at Futaojima Island (locality no.4, Table1). Pupal attendance has also been observed previously. *Jamides celeno* pupae remain in attendance by *Camponotus (Tanaemyrmex)* sp. in protective chambers or byres constructed by the ants from soil and leaf debris (Eastwood *et al.*, 2005).

During a field trip at Ainoshima Island (locality no.5, Table1), one wounded larva was found overwhelmed and attacked by a group of *C. nawai*, although other larvae were being tended nearby (Fig. 2d). It seems that such behavior has been little reported so far. One of the reported cases is in the larva of *Surendra quercetorum discalis*. The larva parasitized by a dipteran has been noted for being eaten by its attendant ants; this took place immediately after the dipteran had eclosed and left a hole in the larval body (Van der Poorten and Van der Porten, 2013). In most cases, ants treat caterpillars as a source of food and immediately attack when the larvae approach. However, lycaenid caterpillars can influence or manipulate the behavior of ants by suppressing ant-aggressiveness (Fiedler, 1991). Any caterpillars that fail to appease or deter the encountered ants, such as by delivering nutritious droplets from DNO or secreting volatile substances, liked the wounded larva in our case, probably have a low chance of survival.

Almost all members of the Polyommataini with known life histories are myrmecophilous (Fiedler, 1991). Within

Table 2. List of attendant ants that were observed associating with late instar larvae of *Tongeia fischeri*. Locality details are given in table 1.

Attendant ant		Locality							
Subfamily	Species	Tojushi	Are	Wanura	Futaojima	Ainosima	Odono	Yamakuni	Yabakei
Dolichoderinae	<i>Ochetellus glaber</i> (Mayr, 1862)			×					×
Formicinae	<i>Lasius productus</i> Wilson, 1955	×							
Myrmicinae	<i>Cardiocondyla nuda</i> (Mayr, 1866)								×
	<i>Crematogaster nawai</i> Ito, 1914		×		×	×	×	×	
	<i>Pheidole fervens</i> Smith, 1858								×
	<i>Tetramorium bicarinatum</i> (Nylander, 1846)								×
	<i>Tetramorium tsushimae</i> Emery, 1925						×		×

Japanese Polyommata species, four species are obligate, whereas the others are facultative, ranging from casual and unstable, through regular to nearly permanent ant-associations (Fukuda *et al.*, 1984; Fiedler, 1991). A number of eggs and larvae of *T. fischeri* that were brought back to the laboratory pupated and successfully became adults in the absence of ants. These results, in addition to the nonspecific ant-association of *T. fischeri*, confirm that the butterfly is a facultative myrmecophile as previously suggested by Fiedler (1991). The future direction of this study will be to survey many other localities covering areas of *T. fischeri* distribution, such as Shikoku and Honshu, in order to increase knowledge of the life history and ant-association in this butterfly.

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摘要

クロツバメシジミ幼虫の好蟻性器官とアリとの関連性(シジミチョウ科: ヒメシジミ族)(Ekgachai JERATTHITIKUL・Naratip CHANTARASAWAT・矢後勝也・疋田 努)

シジミチョウ科の幼虫は、アリと単なる共存から片利共生、相利共生、また共生の中でも特定のアリとしか結び付かない絶対的共生から多くの種のアリと関係する任意的共生、さらには寄生(アリ幼虫の捕食)に至るまでの広範な関係を持つことが知られる。一部ではアリと共生する半翅目を捕食したり、その分泌物を食して成育するものもある。これらのアリに襲われずに共生等の関係を続けられる性質は好蟻性(myrmecophily)と呼ばれる。そしてアリとの相互関係を維持できる基盤には、アリの制御を可能とする化学的、音響的あるいは視覚的信号をつかさどる好蟻性器官(myrmecophilous organs)の存在が重要となる。

シジミチョウ科ヒメシジミ族に属するクロツバメシジミは、国内では東北地方を除く本州から四国、九州にかけて局地的に分布するシジミチョウ科の一種である。本種の幼虫もアリとの関連性がすでに知られているが、その好蟻性や好蟻性器官に関する詳しい情報はこれまであまり知られていない。2009年から2011年にかけて、筆者らは九州地方の9カ所において本種の調査を行い、幼生期を含む本種を観察、採集した他、幼虫の好蟻性や随伴するアリ類などに関するいくつかの知見も得た。

採集した幼虫の体表に見られる好蟻性器官に関してSEMを用いて調べたところ、一般的な共生関係が知られるシジミチョウ科幼虫が持つ基本的な3つの好蟻性器官、すなわち蜜腺(DNO = dorsal nectary organ)、伸縮突起(TOs = tentacle organs)、PCOs(Pore cupola organs)が認められた。

蜜腺は腹部第7節の背中域に見られる横長に開口した大きな器官で、ここからアリが好むアミノ酸や糖類を含む分泌物を多量に放出することが知られる。

伸縮突起は腹部第8節の背側域に備える一対の伸縮可能な筒状器官で、本種では先端部周辺に20前後の羽毛状の突起を備えていた。この器官からアリの行動を制御する揮発性物質が放出されるとも言われるが、単に物理的(あるいは視覚的)に刺激をアリに与える器官かもしれず、詳しい機能は不明である。

PCOsはドーム形または多少凹んだレンズ状の上部を備え

た円柱形の微小器官で、体表全体に散在するが、特に本種では蜜腺と気門の周囲に多く見られた。本種のPCOsは側面上部に4~8つの三角状の短い突起を有し、特にこの形状はツバメシジミ類の近縁種 *Cupido minimus* と酷似し (Baylis and Kitching, 1988), その類縁性がうかがえる。この器官の表面からアリの体表物質に類似した組成の炭化水素やアミノ酸などが検出されるために分泌器官の一つとされる。

その他の好蟻性器官として樹状突起 (dendritic setae) が認められた。樹状突起はDNOの周辺や気門の周囲、前胸背楯板上などによく生じるが、本種ではDNOの両側の周囲のみに限られていた。DNOやTOsを持たない好蟻性の種の体表上にも散見されることや、物理的な刺激に対する受容器として機能することなどから、アリを感知する重要な感覚毛とされる。

好蟻性器官以外の注目すべき構造として、体表全体に散在

する刺毛が通常の針状の他にやや扁平なしゃもじ状となるものも少なからず見られた。この形状は好蟻性との関連性によるものと考えられる。また、ソケットの多くは星形をしていたが、これはヒメシジミ族に広く見られる形状である。

さらに今回の調査では、幼虫に随伴するアリとしてルリアリ、ヒゲナガケアリ、ハダカアリ、ツヤシリアゲアリ、ミナミオオズアリ、オオシワアリ、トビイロシワアリの7種を記録した。このうちトビイロシワアリを除く6種は、本種の共生アリとして初記録の可能性がある。このように複数種のアリ類との関連が確認された結果から、おそらくクロツバメシジミの好蟻性は“任意的共生関係”と考えられるが、今後は九州以外の本州から四国にかけての他地域での好蟻性や共生アリなどの調査も必要であろう。

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